Metabolic Alkalosis, Bedside and Bench

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Although significant contributions to the understanding of metabolic alkalosis have been made recently, much of our knowledge rests on data from clearance studies performed in humans and animals many years ago. This article reviews the contributions of these studies, as well as more recent work relating to the control of renal acid-base transport by mineralocorticoid hormones, angiotensin, endothelin, nitric oxide, and pottasium balance. Finally, clinical aspects of metabolic alkalosis are considered.
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I t is always interesting to observe how many of the young physicians interviewing for nephrology fellowship positions report that their interest in the field is based on a personal delight in the complexities of acid-base and electrolyte problems. It is with sadness that we explain to them that (1) consulting nephrologists, whether in private practice or in an academic setting, receive few, if any, requests to evaluate acid-base or electrolyte problems, and that (2) their (the applicants) dreams of a career in clinical research investigating acid-base physiology probably would go unfulfilled in the absence of significant basic skills in molecular biology and a willingness to spend large amounts of time at the bench and an even greater amount of time dealing with regulation and bureaucracy.

The situation was considerably different in the decades of the 1960s and 1970s, when a great number of significant clinical studies of acid-base physiology were performed. The techniques of the beginning of that era were astute history taking, careful physical examination, arterial and/or venous blood gas determination, acid and alkali titration of blood and urine, and clinical chemistry. The designs of choice were the balance study and the clearance study. Virtually any graduate of an accredited medical school was equipped to contribute to the research effort. No long period of additional special training was required. Those with an interest and a keen intellect could readily participate. An additional aspect of this period is that the research proceeded in such a way that the clinical answers sometimes came first. Groundbreak-ing clinical studies in many cases defined the human condition in acid-base disorders earlier than the animal studies that outlined the underlying physiology.

Early Studies Relating to Metabolic Alkalosis

The outstanding studies of Pitts et al[1,2] outlined for nephrologists the integrated result of the renal transport of bicarbonate in the human being before the diverse mechanisms by which bicarbonate transport occurred were ever elucidated in animal models. These investigators infused themselves with bicarbonate to achieve increasing serum bicarbonate concentration and carefully quantified the resulting urinary bicarbonate excretion. The results defined a curve for renal bicarbonate handling in the human being that displayed a steady increase in reabsorption until it reached its maximum when the serum bicarbonate concentration exceeded 28 mmol/L. The kidney then maintained an apparent maximal rate of absorption, or tubular maximum (Tm); the further infusion of bicarbonate only increased the rate at which bicarbonate escaped the kidney and was excreted. These studies defined a relationship between bicarbonate load and reabsorption, and carry within them the seed of the relationship between volume and the maintenance phase of metabolic alkalosis, that is, the kidney can support and maintain increased blood bicarbonate concentration if the product of the glomerular filtration rate and the bicarbonate concentration is less than the Tm for bicarbonate.

Cohen[3] performed a critical study of metabolic alkalosis in 1968 when he examined the effect of volume expansion with isometric fluids (defined by having concentrations of so-

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dium, chloride, bicarbonate, and potassium identical to that already present in the blood) in dogs with metabolic alkalosis induced by diuretics and a low-chloride diet. In this classic balance study, Cohen[3] showed that replacement of lost volume corrected an increased blood bicarbonate concentration, even when fluid with a bicarbonate concentration that did not differ from that seen in the alkalemic animals was administered. Thus, as volume was administered, the kidney responded by adjusting the composition of the blood to return the acid-base state to normal.

It was in this context that Dr. Neil Kurtzman[6] published an article that can reasonably be said to have launched his academic career. While working at the Metabolic Burn Unit of the Brooke Army Medical Center under the guidance of Colonel Basil Pruitt, MD, Dr. Kurtzman developed an intense interest in the problems of the renal response to perturbations of acid-base balance and of volume. In particular, the relationship between extracellular volume, specifically "the effective arterial blood volume" and the renal handling of bicarbonate became his question of the day. Clinical observations already had been made. The administration of sodium chloride was known to increase the excretion of bicarbonate in patients, but whether this was caused by chloride itself, by the effect on blood pressure, by the sodium that accompanied the chloride, or by some other process was not certain. Kassirer et al[4,5] already had outlined the clinical treatment of metabolic alkalosis: provision of chloride, and, in addition, correction of hypokalemia when deficits were serious. Thus, the physician of that day did not know exactly what went on in the black box that was the kidney, but recognized the conditions that cause metabolic alkalosis and what needed to be performed to correct it. In short, although the cause and the treatment of metabolic alkalosis were known, the debate concerned why treatment worked.

In the seminal paper of 1970, Kurtzman[6] investigated how dogs responded to the administration of isotonic sodium bicarbonate, isotonic sodium chloride, and hypertonic sodium bicarbonate plus isotonic saline. The results indicated that dogs given hypertonic bicarbonate initially developed an increase of the plasma bicarbonate level (metabolic alkalemia) but rapidly excreted the excess bicarbonate when infused with isotonic saline. The bicarbonaturia occurred in concert with a massive increase in the fractional sodium excretion and an increase in potassium excretion that induced marked hypokalemia. Additional dogs given isotonic saline without prior bicarbonate infusion developed significant bicarbonaturia despite initially normal, and, later, low serum bicarbonate levels. Bicarbonaturia even developed after volume expansion in dogs with low serum bicarbonate induced deliberately with infusion of HCl. Although a Tm seemingly developed at a blood bicarbonate concentration in the 26 to 28 mEq/L range in normal dogs given bicarbonate, the volume-expanded dogs appeared to show a Tm at serum bicarbonate concentrations as low as 16 to 18 mEq/L. Thus, saline expansion increased bicarbonate loss to the urine regardless of the initial bicarbonate concentration; something about the infusion of volume, sodium, or chloride resulted in bicarbonaturia.

The administration of chloride per se is not needed to increase bicarbonate excretion. The volume expansion of dogs with isotonic bicarbonate resulted in a natriuresis (fractional excretion of sodium = 16.8%), bicarbonaturia, and depressed the reabsorption of bicarbonate. By contrast, dogs given bicarbonate after induction of absolute volume contraction by hemorrhage increased their rate of bicarbonate reabsorption and developed a markedly increased serum bicarbonate concentration. Similar results were obtained when dogs were given bicarbonate in the presence of ligation of the thoracic vena cava, another experimental model marked by a severe decrease in effective arterial blood volume. Both the hemorrhage group and the caval ligation group showed low sodium and chloride excretion. When the caval ligation was released and effective arterial blood volume was restored, sodium and chloride excretion increased rapidly, and bicarbonaturia developed.

The earlier-described results were interpreted by Dr. Kurtzman[6] to indicate a relationship between bicarbonate reabsorption and effective arterial blood volume, and the groups exposed to isotonic sodium bicarbonate infusion showed that bicarbonate excretion could be increased without the infusion of chloride. Kurtzman[6] further showed that the infusion of sodium bicarbonate did not increase bicarbonate or sodium excretion if volume expansion was prevented.

The clearance studies by Kurtzman[6] in dogs and the contemporaneous studies of Purkerson et al[7] in rats clearly established that there was a relationship between bicarbonate reabsorption and volume; and, by extension, explained the previously observed response of metabolic alkalosis to the infusion of saline. Kurtzman[8] further linked the increase in bicarbonate reabsorption observed in acute hypercapnia to changes in blood pressure, effective arterial blood volume, glomerular filtration rate, and filtered load of bicarbonate. When blood pressure and filtered load were supported, the increase in bicarbonate reabsorption that accompanied hypercarbia was grossly blunted.

These observations were confirmed and extended as physiologic techniques increased in sophistication. The studies of bicarbonate transport performed after re-emergence of renal micropuncture in several laboratories,[9-13] and the efforts of McKinney and Burg[14] and Burg and Green[15] to develop in vitro tubular microperfusion allowed the careful dissection of renal tubular reabsorption. In vivo micropuncture studies established that bicarbonate reabsorption was almost exclusively a proximal tubule phenomenon with high capacity. Alpern and Cogan et al[16-20] used micropuncture and the further refinement of in vivo microperfusion to redefine the Tm in terms of maximum rates of reabsorption by subsegments of the proximal tubule, passive reabsorptive processes related to concentration gradients and solvent drag, and back leak of bicarbonate from the blood to the tubule lumen as intraluminal bicarbonate concentrations decreased.[21] Bank et al[22] first suggested that the earliest portion of the proximal tubule, the S1 segment, contained a proton pump (an H-adenosine triphosphatase [ATPase]), a hypothesis later confirmed definitively as molecular biologic techniques

Table 1 Factors Controlling Proximal Bicarbonate Reabsorption

Upregulating Factors	Downregulating Factors
↓ peritubular [HCO$_3$], pH	↑ peritubular [HCO$_3$], pH
↑ peritubular CO$_2$	↓ peritubular CO$_2$
Chronic acidosis (systemic)	Volume expansion (systemic)
↑ luminal [HCO$_3$]	Parathyroid hormone
↑ luminal fluid flow rate	
↑ HCO$_3$ delivery	
All	
Catecholamines	
Dopamine	
Insulin	
Glucocorticoid hormone	
ET-1	
↓ peritubular [K]	
Potassium depletion	
Hypercalcemia	

were applied to the question.[23] The greater portion of proximal tubule bicarbonate absorption in the S2 segment was shown to be sodium dependent and mediated by a brush-border membrane sodium-proton exchanger, later defined as the NHE3 antiporter, one of a large family of sodium-proton exchangers.[24,25] Beyond the reach of in vivo techniques, the S3 segment was found to again have a proton pump, but reabsorptive capacity was balanced by increased back leak in this segment. The final bicarbonate concentration achieved at the end of the proximal tubule, which is inaccessible to in vivo techniques, has been estimated to be approximately 7 to 8 mmol/L.

When the control of proximal nephron bicarbonate reabsorption was investigated with micropuncture and microperfusion, multiple critical factors were identified, including the effects of extracellular volume, bicarbonate delivery, peritubular bicarbonate and pH, carbon dioxide tension, tubular fluid flow rate, angiotensin II (AII), catecholamines, insulin, parathyroid hormone, and other hormones.[25] The modulators of proximal tubule bicarbonate reabsorption are listed in Table 1.

The advent of microperfusion techniques also has permitted the evaluation of acid-base transport in the loop of Henle. The mechanisms found in the loop include Na/H exchange and ammonium transport; in addition, the H-ATPase is found in the apical membrane of cells in the thick ascending loop. When bicarbonate and inulin concentrations at the end-accessible superficial proximal tubule and the early distal convoluted tubule (DCT) are compared, 10% to 20% of the bicarbonate leaving the proximal tubule is found to be reabsorbed. However, micropuncture and in vivo microperfusion do not permit one to determine the quantitative contribution of the proximal straight tubule versus the thick ascending loop with regard to this reabsorption. In vivo microperfusion studies performed in rats with metabolic acidosis show increased bicarbonate reabsorption between the end proximal and

early distal tubule; reabsorption also was increased in response to acute but not chronic metabolic alkalosis.[26,27] Good[28] studied the medullary thick ascending loop using in vitro microperfusion and found that both in vivo metabolic acidosis and in vivo bicarbonate loading of the rats from which tubules were obtained increased the reabsorption of total CO$_2$ and ammonium by this segment. The role of this segment in generation and maintenance of metabolic alkalosis thus remains open to question.

The physiology of the DCT and the collecting duct in renal acid-base transport and metabolic alkalosis is complex. The earliest studies that localized a decrease in pH to the collecting duct were performed by Ullrich and Eigler,[29] using a microcatheter advanced up the papilla to obtain samples in the golden hamster. That the distal nephron and collecting duct system had a role in acidifying the urine were confirmed further by measurement of transport in the distal tubule, and also could be imputed from the pH and bicarbonate of the final urine relative to values at the most distal accessible portion of the DCT.

The distal nephron, including the collecting duct system, was identified clearly as a major contributor to bicarbonate handling as microtechniques permitted the segmental dissection of renal transport. Studies over the past 20 years defined the DCT and the cortical collecting duct as sites of great adaptability of bicarbonate transport. The DCT, a complex mosaic of cells with a wide variety of physiologic capacity, appears to both secrete and reabsorb bicarbonate at all times, with alteration of what is generally net bicarbonate reabsorption as a result. The early distal tubule appears to secrete protons by a sodium-proton exchange mechanism (NHE2), the late distal tubule may show the H,K-ATPase pumps, and the H-ATPase is seen throughout the segment.[30-32] Bicarbonate secretion occurs in the later portions of the distal tubule.[33-35] Careful dissection of the unilateral vectors of bicarbonate movement under a variety of acid-base conditions by Wesson[32] and Wesson and Dolson[34,36] has shed some light on this difficult segment, with the most important recent development being the identification of endothelin, a hormone involved in regulation of blood pressure and volume responses, as a significant modulator of bicarbonate transport. Bicarbonate loading served to increase bicarbonate secretion without decreasing proton secretion; when chloride was excluded from the luminal fluid, proton secretion was found to be enhanced in chronic metabolic alkalosis.[34,37] Acid loading of animals reduced the rate of bicarbonate secretion without increasing the rate of proton secretion.[38] Studies also showed that endothelin concentrations increase during acid loading, that endothelin stimulates the rate of acid secretion and thus shifts the balance of transport to net reabsorption. Inhibitors (ET1b) of endothelin blunt the response to acidosis.[36] More recently, examination of bidirectional bicarbonate flux in rats with chronic alkalosis has indicated that in this model, there is increased activity of the Na-H antiporter and the H,K-ATPase in the DCT, but no change in the activity of the H-ATPase.[32]

Both net bicarbonate reabsorption and net bicarbonate secretion have been observed in the collecting duct, depending

on the pretreatment of the experimental animal.[39-41] Bicarbonate secretion in this segment appears to be sensitive to cyclic adenosine monophosphate (cAMP)-mediated stimuli in the short term and was affected by chloride presence in the lumen and chloride gradients across the epithelium.[42,43] Bicarbonate reabsorption mediated by proton secretion by an H-ATPase and an H,K-ATPase also varied, with control of the H-ATPase by aldosterone and control of the H,K-ATPase by potassium balance.[44-46] In the long term, the adaptation of both proton-secreting type A intercalated cells and bicarbonate-secreting type B intercalated cells appeared to play a significant role. Whether such cells merely undergo a shift between activated and quiescent states or actually totally shift polarity from proton secretion to bicarbonate secretion has never been resolved completely. The deeper collecting duct segments show only proton secretion. The medullary collecting duct responds to mineralocorticoid hormone by increasing H-ATPase activity, and to potassium depletion by increasing H,K-ATPase activity.[46] Table 2 outlines the known modulators of acid-base transport in the DCT and the collecting duct system.

The importance of the collecting ducts in metabolic alkalosis lies in the response of these segments to mineralocorticoid hormone. Desoxycorticosterone acetate was noted to induce an increase in serum bicarbonate concentration in

human beings 50 years ago.[47] However, in the absence of other confounders, the effect of mineralocorticoid on serum bicarbonate is mild. The administration of exogenous aldosterone results in only a 1 to 3 mEq/L increase in serum bicarbonate level.[48] On the other hand, an increase of mineralocorticoid hormone levels increases urinary potassium excretion and thus contributes to potassium depletion, a major influence in metabolic alkalosis.

Clinical Relevance of Metabolic Alkalosis

There is no accurate estimate of the incidence or prevalence of metabolic alkalosis. To quote many, metabolic alkalosis is not an overwhelming issue in public health. Most patients who have mild acute or chronic metabolic alkalosis are not hospitalized; even fewer of these individuals are ever specifically diagnosed. Most patients with metabolic alkalosis never have the requisite laboratory testing, which includes an arterial blood gas measurement, to make a certain diagnosis. Certainly the only estimates of epidemiology that can be generated would need to be based on the frequency of metabolic alkalosis as an inpatient diagnosis. Our ability to generate accurate estimates of secondary diagnoses was lost long ago

Table 2 Factors Regulating Distal Nephron Acidification of the Urine

	Upregulating Factors	Downregulating Factors
DCT		
[HCO$_3$] secretion	↑ luminal flow rate	↓ luminal flow rate
	Glucagons	ET-1
	Prostacyclin	
	Metabolic alkalosis	
[H] secretion by Na/H exchanger	ET-1	
[H] secretion by H,K-ATPase	K depletion	
	Metabolic alkalosis	
CCD		
[HCO$_3$] secretion	Catecholamines	↓ luminal [Cl]
	↓ luminal [HCO3]	↑ luminal [HCO$_3$]
	↑ luminal [Cl]	Metabolic acidosis
	Metabolic alkalosis	
	Mineralocorticoid hormones	
H-ATPase–mediated H+ secretion	Mineralocorticoid hormones	↓ peritubular CO$_2$
	↑ peritubular CO$_2$	↑ peritubular [HCO$_3$]
	↓ peritubular [HCO3]	↓ buffer delivery
	buffer delivery	↓ Na reabsorption, lumen −PD
	↑ Na reabsorption, lumen −PD	metabolic alkalosis
	↓ luminal pH	
	metabolic acidosis	
H,K-ATPase–mediated H+ secretion	Potassium depletion	Potassium excess
MCD		
H-ATPase–mediated H+ secretion	Mineralocorticoid hormones	↓ peritubular CO$_2$
	↑ peritubular CO$_2$	↑ peritubular [HCO$_3$]
	↓ peritubular [HCO$_3$]	Metabolic alkalosis
	↓ luminal pH	
	Metabolic acidosis	
H,K-ATPase–mediated H+ secretion	Hypokalemia,	Hyperkalemia,
	Potassium depletion	Potassium excess
		↓ luminal potassium

when discharge diagnosis lists became irretrievably enmeshed with billing issues. A patient who develops metabolic alkalosis as a result of diuretic administration for the treatment of congestive heart failure and vomiting related to liver dysfunction consequent to passive congestion may be discharged with diagnoses of congestive heart failure, passive congestion of the liver, emesis, and arrhythmias, but will not likely bear diagnoses of contraction of effective arterial blood volume, hypokalemia, potassium depletion, or metabolic alkalosis. The Center for Medicare and Medicaid Services limits the number of diagnoses to be reported. Billing clerks routinely look for the most lucrative diagnoses and the Disease Related Group payment for metabolic alkalosis is not lucrative. Furthermore, if metabolic alkalosis is a result of prior treatment with diuretics, emesis as a result of medications, or inattention to volume status then the diagnosis may be viewed as a red flag for potential lawsuit, and the attention it would bring could prevent one from highlighting its occurrence.

When one considers the causes of metabolic alkalosis listed previously, it is obvious that many patients (all those who develop metabolic alkalosis after nasogastric suction, those on diuretics, and those developing posthypercapnic alkalemia) have the disorder on the basis of therapeutic misadventure. To these also must be added the growing incidence of alkalosis resulting from the use of citrate anticoagulation during chronic renal replacement therapy, and alkalosis caused by the increasing use of calcium carbonate to treat bone disorders in the aging. The exact percentage of cases of metabolic alkalosis that are iatrogenic remains unknown.

Alkalosis and alkalemia are important because they alter morbidity and mortality. Increase of blood pH increases cardiac arrhythmia, probably through mechanisms involving a decrease in ionized calcium concentration and potassium shifts.[49] Other effects include alteration of consciousness, increased seizure activity, decreased oxygen release to tissue from hemoglobin, tetany secondary to hypocalcemia, increased ammonia generation by the kidney, and, in some instances, depression of the respiratory drive.[50-53] Although the data regarding this issue are far from plentiful, Luke[54] recently reviewed in detail the incidence and impact of metabolic alkalosis. Metabolic alkalosis has been reported to account for more than half of all acid-base disorders in an intensive care setting, and also was associated with high mortality rates.[55-57] Because mortality is especially high when a pH in excess of 7.6 develops, intervention at a pH of 7.55 and greater has been recommended.[58] Data regarding hospitalized patients outside the intensive care setting are not available and likely will never be forthcoming.

Metabolic Alkalosis in Clinical Practice

It is useful to begin a discussion of clinical metabolic alkalosis with a precise definition of terms, even when one presumes a readership with significant knowledge of medicine and ne-

phrology. The clinical term *alkalemia* defines a pathophysiologic state in which there is an observed increase in the pH of the blood that is greater than normal. By contrast, the term *alkalosis* here defines a pathophysiologic process that tends to increase the relative amount of base, or alkali, in the body. In the case of metabolic alkalosis, the pathophysiologic processes that tend to increase the net amount of alkali in the body are either the loss of fixed acid derived from metabolic sources to the external environment, or the ingestion of base. Because any number of acid-base abnormalities may co-exist in a single patient, the presence of alkalosis does not presume that alkalemia also is present. For instance, a patient with metabolic alkalosis may have an ongoing state of metabolic acidosis, as in the case of a patient who initially has metabolic alkalosis caused by vomiting, and then develops diabetic ketoacidosis as a result of a failure to take insulin. If the ketoacidosis is severe, the arterial blood pH may be well below normal, but the initial metabolic alkalosis still would be present.

Regardless of differences in opinion arising from the interpretation of study results relative to the relative importance of volume, chloride, and potassium, it generally is agreed that metabolic alkalosis is divisible into 2 phases, a *generation phase* during which the relative concentration of alkali within the body increases or tends to increase, and a *maintenance phase* during which the increased bicarbonate is retained. These 2 phases are each considered later.

The Generation of Metabolic Alkalosis

Excess Alkali Administration or Intake

The simplest way to increase serum bicarbonate concentration is to administer excessive amounts of base to the patient. Although this is never performed purposefully to create metabolic alkalosis, several common clinical errors by patients or physicians may produce this result. The milk-alkali syndrome once was seen commonly when peptic ulcer disease was treated with calcium carbonate and the Sippy diet.[59] The frequency at which it was diagnosed decreased markedly with the introduction of H2 blockers and H,K-ATPase inhibitors, especially since the recognition of *Helicobacter pylori* as the cause of ulcer led to the development of curative triple-drug therapy. However, the number of cases may be increased most recently because of the emphasis on the use of dairy and calcium supplementation for prevention or the treatment of osteoporosis and, recently, the promotion of weight loss, especially if vitamin D also is taken. The condition is more likely to develop if renal insufficiency is present.[60]

Excess administration of base also may arise as a result of overtreatment of metabolic acidosis resulting from administration of bicarbonate in the treatment of diabetic ketoacidosis or lactic acidosis, and the misinterpretation of the depressed serum bicarbonate in respiratory alkalosis as a sign of metabolic acidosis in the absence of measurement of arterial pH. Finally, the introduction of citrate anticoagulation in

chronic renal replacement therapy has led to the occurrence of metabolic alkalosis as a result of the large quantities of bicarbonate precursor during treatment[61,62] If completely converted, each millimole of citrate given produces 3 mmol of bicarbonate. If adjustments are not made in replacement fluids and dialysate, severe metabolic alkalosis may develop, even in patients with renal failure.

Gastric Alkalosis

Gastric loss of acid is a common cause of metabolic alkalosis. It may result from emesis or from the use of nasogastric suction or drainage. The concentration of acid in gastric juice is significant; the pH generally is less than 2.0. A liter of gastric juice at a pH of 1.0 has a proton concentration equaling 100 mEq/L; up to 4 L/d of gastric juice may be lost to suction in the presence of outlet obstruction. Lesser amounts are lost if some of the stomach's output reaches the duodenum to react with secreted bicarbonate. The maximum loss of acid by this route is therefore 400 mEq/d of acid. Notably, because it is hydrochloric acid that is lost, this involves the loss of up to 400 mEq of chloride. Thus, in the presence of severe vomiting or continuous suction, significant volume loss accompanies the ongoing loss of acid. If volume loss is not monitored carefully and replaced appropriately, significant volume depletion will occur, triggering the secretion of aldosterone.

Gastric alkalosis almost always is associated with the development of potassium depletion, but the potassium loss is not a direct consequence of emesis. Rather, the increase of mineralocorticoid levels in response to volume depletion stimulates renal potassium secretion at the distal nephron and collecting duct.

The risk of developing gastric alkalosis can be minimized by the use of antiemetics when indicated, limiting the use of nasogastric suction, by careful attention to volume losses and appropriate replacement of ongoing losses, and by reduction of gastric acid secretion in the presence of nasogastric suction.[63] In the absence of concurrent use of diuretics, the urine chloride can be used to determine when the volume state returns to normal.

Diuretic-Induced Alkalosis

That diuretics may induce metabolic alkalosis has been realized since the introduction of effective diuretic agents over a half a century ago. Loop diuretics and thiazides have 3 important effects relative to the generation of metabolic alkalosis. First, they increase acid excretion by the kidneys. Net acid excretion increases markedly in response to both types of diuretics. Loop diuretics also decrease urine pH, indicating increased acid excretion, a property used in the furosemide test for renal tubular acidosis.[64] Because both loop diuretics and thiazides work beyond the proximal tubule, they have little direct effect on bicarbonate reabsorption. However, both types of diuretics induce chloriuresis and natriuresis. In addition, the loop diuretics, which inhibit the Na-K-2Cl cotransporter of the loop of Henle, directly increase potassium urinary potassium loss.

The volume depletion that develops in response to diuretic use results in the stimulation of aldosterone secretion. Again, aldosterone, by increasing apical membrane sodium permeability and the basolateral membrane Na,K-ATPase activity in principal cells of the cortical collecting duct and in distal nephron cells, causes an increase in the luminal negativity of these segments. Potassium secretion, a passive process responsive to the epithelial potential, is increased. In addition, this passive secretory process is sensitive to the rate at which tubular fluid flows past the collecting ducts. As diuretics increase flow, potassium secretion increases directly.

Other diuretic agents have opposing effects. Although rarely used as diuretics per se, carbonic anhydrase inhibitors effectively limit the reabsorption of bicarbonate in the proximal tubule and also inhibit acid secretion in the distal nephron and collecting duct. Thus, their use routinely results in the development of hypobicarbonatemia and metabolic acidosis. However, by increasing distal delivery of sodium, increasing the delivery of a nonreabsorbable anion (bicarbonate is nonreabsorbable in the distal nephron after carbonic anhydrase inhibition), and increasing flow rate, carbonic anhydrase inhibitors can cause significant potassium wasting. This is of some importance when these agents are used to induce diuresis and bicarbonate loss in patients with so-called posthypercapnic metabolic acidosis.

Spironolactone, eplerenone, amiloride, and triamterene all reduce acid excretion and potassium loss by interfering with mineralocorticoid action in the distal tubule and the collecting duct. These agents are not associated with metabolic alkalosis, rather they tend to induce a mild hyperchloremic metabolic acidosis.

Primary Mineralocorticoid Excess

Aldosterone and other adrenal hormones with mineralocorticoid activity stimulate sodium reabsorption in the DCT and the cortical collecting duct by increasing the apical membrane sodium permeability of principal cells and increasing Na,K-ATPase activity in these cells as well. As sodium reabsorption increases in these segments via the apical membrane sodium channel (ENaC), there is a resulting increase in the lumen negative electrical potential across these epithelia. The negative potential increases the passive transfer of potassium into the tubule lumen, producing a kaliuresis. In addition, the lumen negativity reduces the work required for active proton secretion by the electrogenic H-ATPase found in both sites. Proton secretion subsequently is enhanced.

In addition to stimulating the Na,K-ATPase, aldosterone also stimulates the electrogenic H-ATPase. The rate of proton transport increases, but the power of the pump does not change, suggesting that more pumps are made available in the membrane. H-ATPase is increased in the medullary collecting duct and the previously mentioned segments.

There are several syndromes of primary mineralocorticoid excess associated with hypokalemia and alkalosis. These include primary aldosteronism (Conn's syndrome, adrenal hyperplasia, and adrenal carcinoma), the adrenogenital syndrome (11β- and 17α-hydroxylase deficiency),

the syndrome of apparent mineralocorticoid excess, the syndrome of glucocorticoid-remediable hyperaldosteronism, and familial hyperaldosteronism type II. Hypokalemia and alkalosis may be seen as well in secondary states of hypermineralocorticoidism unassociated with volume depletion (unilateral renal artery stenosis, tumor of the juxtaglomerular apparatus).

Primary Glucocorticoid Excess

Adrenal hyperactivity, whether primary or secondary, has long been associated with metabolic alkalosis. Several mechanisms appear to be involved.

Although mineralocorticoid hormones have been shown to stimulate acid secretion in the collecting duct, glucocorticoid hormones appear to exert primary effects on the proximal tubule and loop of Henle.[65-68] Both Na/H exchange and Na,K-ATPase activity appear to be stimulated by these hormones; theoretically the proximal reabsorption of volume and bicarbonate should be increased. However, the direct administration of glucocorticoids does not result in the development of metabolic alkalosis.[69] This being said, why do patients with Cushing's disease develop metabolic alkalosis? Most do not have increased aldosterone levels. However, nonaldosterone mineralocorticoid hormones are found in a significant number, and glucocorticoid hormones do exert mineralocorticoid-like effects.[70-72] The mineralocorticoid-like effects include not only increased acid excretion, but also increased potassium secretion and the development of potassium depletion.

Renal Tubular Disorders Associated With Alkalosis and Potassium Wasting

Several renal tubular disorders are associated with both potassium wasting and metabolic alkalosis. These include Bartter syndrome, Gitelman's syndrome, and Liddle syndrome.

Bartter syndrome is characterized by the presence of profound hypokalemia, metabolic alkalosis, volume depletion with low blood pressure, and the development of renal failure caused by tubulointerstitial scarring. Its cause was long a mystery. The metabolic aspects of the disorder resembled mineralocorticoid excess, but blood pressure was low and volume depletion could be severe. Kurtzman and Guitierrez[73] once suggested that a chloride leak was the most likely cause because of the resemblance of the condition to surreptitious use of loop diuretics with secondary hyperaldosteronism. The true nature of the defect in Bartter syndrome was not understood until after the mechanisms of chloride, sodium, and potassium reabsorption in the loop of Henle was defined.[74,75] Recent studies have indicated that the disorder may result from any one of several genetic defects involving the following: (1) the Na-K-2Cl cotransporter in type I,[76] (2) the luminal potassium channel (ROMK) in type II,[77] (3) the basolateral membrane chloride channel (CLCNKB) in type III,[78] and, (4) a subunit of the Cl channel called *barttin* in type IV.[79] The type IV patients also are afflicted with sensorineural deafness. Although the individual mechanisms differ, in every instance the result is potassium loss with volume depletion.

In type I it occurs by direct inhibition of Na, K, and Cl transport and in type II it is caused by secondary failure of the cotransporter consequent to a primary defect of potassium recirculation. In types III and IV the failure of basolateral chloride exit results in the secondary failure of Na-K-2Cl uptake. The volume depletion that follows the sodium chloride loss induces aldosterone secretion, which results in further acid and potassium secretion by the collecting duct.

Gitelman's syndrome closely resembles Bartter syndrome but it also is associated with the presence of significant urinary magnesium wasting and hypomagnesemia. Gitelman's syndrome also presents with hypercalcemia and hypocalciuria. It has been shown to be caused by any one of several defects affecting the thiazide-sensitive Na-Cl cotransporter of the distal tubule.[80,81] The genetic defect thus clearly explains the similarity of the disease physiology to the effects of thiazide diuretics. The failure of NaCl cotransport results in continual natriuresis, chloruresis, and volume depletion. There is subsequent aldosterone release and further potassium loss, and urinary proton secretion follow. Hypercalcemia and hypocalciuria are a consequence of inhibition of NaCl uptake after thiazide diuretic use. The mechanism of magnesium loss is not well understood.

Not all cases of Bartter and Gitelman's syndromes are clearly distinguishable, and additional genetic defects have been located with syndromes closely resembling each.[82] If the clinical phenotype strongly suggests one or the other syndrome is present, the absence of the expected genotype should lead to a search for additional defects rather than the abandonment of the diagnosis.

Liddle syndrome more closely resembles pure mineralocorticoid excess than either Bartter or Gitelman's syndromes. In Liddle syndrome metabolic alkalosis and potassium deficiency is associated with hypertension in the presence of low aldosterone levels. Molecular genetic studies defined the cause of Liddle syndrome to be an abnormal β subunit of the distal nephron ENaC that causes the channel to remain in its open configuration for a greater percentage of time than normal.[83] The effect is thus to mimic mineralocorticoid stimulation of sodium entry at this site. Potassium wastage and excessive proton secretion result from increased sodium reabsorption and transepithelial potential. The effect of the increased luminal negativity is enhancement of passive potassium secretion, and also increased proton secretion.

Hereditary Chloride-Losing Diarrhea

Most nephrologists have heard of this entity but very few have ever seen it. Children with the disorder lack normal Cl-bicarbonate exchange in the ileum, resulting in continuous loss of chloride in the stool.[84] Volume depletion results and there is a compensatory increase in aldosterone secretion with subsequent effects on potassium handling producing hypokalemia. It has been treated classically by replenishing volume and potassium losses. More recently, proton pump inhibitors have been advanced as therapy.[85]

Tubular Adenoma

Metabolic alkalosis resulting from secretion of electrolytes by a villous adenoma of the colon was first reported in 1961.[86] These otherwise benign tumors secrete varying amounts of chloride and bicarbonate, and generally secrete sodium and potassium at high concentrations. Volume loss may approach 3 L/d. Because volume depletion will increase aldosterone levels and subsequently lead to renal potassium secretion, alkalosis may result even if potassium is not secreted by the tumor. Potassium secretion by the tumor increases the risk of serious metabolic alkalosis.

Licorice Ingestion

Glycyrrhizinic acid is a naturally occurring compound found in licorice. Although it originally was believed to cause excessive potassium secretion by the distal nephron because it functioned as a mineralocorticoid, later studies found glycyrrhizinic acid to be a competitive inhibitor of 11β-hydroxysteroid dehydrogenase.[87] Carbenoxolone appears to share this behavior.

Potassium Depletion

Renal potassium transport and renal acidification of the urine interact on multiple levels. Potassium depletion and excess alter aldosterone release. Mineralocorticoid stimulation of the distal nephron and collecting duct increases both proton secretion (by increasing H-ATPase activity) and sodium reabsorption. The increased sodium reabsorption increases the lumen-negative transepithelial potential in the cortical collecting duct and thus reduces the work of proton secretion. The increased potential also increases the passive secretion of potassium. Potassium and hydrogen ion transport in the collecting duct also are linked at the H,K-ATPase. Here, obviously, the ions move in opposite directions. The luminal H,K-ATPase is stimulated markedly by potassium depletion, resulting in reclamation of intraluminal potassium, but loss of acid. Unlike the H-ATPase, the H,K-ATPase does not appear to respond to mineralocorticoid hormone directly.

Additional links between potassium homeostasis and acid-base balance exist. Potassium balance is a critical regulator of ammonium metabolism. Potassium depletion stimulates ammonium production and excretion, a response of considerable consequence in patients with liver disease, and a possible mediator of interstitial renal fibrosis in chronic potassium deficiency. Excess potassium and hyperkalemia inhibits ammonium production and excretion, an effect of considerable importance in the management of renal acidosis.

Potassium depletion and hypokalemia affect proximal tubule acidification directly through stimulation of transporters and alteration of intracellular pH as shown by the effects of K depletion on proximal tubules studied in vivo.[88-90] Thus, potassium depletion and hypokalemia may increase proximal reabsorption of bicarbonate and help maintain a state of metabolic alkalosis.

Potassium depletion also has been shown to significantly alter glomerular hemodynamics and it increases renin and AII levels.[90-93] These effects have been implicated in the development of volume-resistant (ie, chloride-resistant) metabolic alkalosis.

Clinical reports indicate that, in human beings, severe potassium depletion results in mild metabolic alkalosis.[94] Both renal and nonrenal mechanisms have been inferred, and the condition is not related to chloride depletion.

Nonreabsorbable Anions

The reabsorption of sodium in the distal tubule and cortical collecting duct is associated with the development and maintenance of a lumen-negative electrical potential that may both increase passive potassium secretion and increase proton secretion by reducing the total gradient against which the H-ATPase pump must work. The electrical potential is the result of the difference between sodium and chloride movement across the membrane. The potential generated by the reabsorption of sodium depends on the conductance of the anion that travels with sodium. (Conductance is the reciprocal of resistance; it is related to chemical permeability and ionic charge.) Substituting an anion less permeable than chloride for chloride increases the electrical potential generated by the transport of sodium. Examples of relatively nonpermeable anions include phosphate and sulfate, which may be used to stimulate acidification of the urine in tests of distal acidification.

Clinically, the substances usually involved in the development of metabolic alkalosis as a result of enhancement of potassium and proton secretion by impermeable anions are the penicillins and cephalosporins.[95,96] The key factors are that the anion is impermeable and that the drug appears in quantity in the urine.

Postmetabolic Acidosis

Metabolic alkalosis may appear in the wake of treated metabolic acidosis as the result of 2 general mechanisms. First, the treating physician may overestimate the amount of bicarbonate required to correct the acidosis. Second, the treating physician may underestimate how much base will be produced from bicarbonate precursors when the underlying cause of overproduction acidosis is corrected in ketoacidosis and lactic acidosis.[97,98] Usually, the metabolic alkalosis will be mild because the additional bicarbonate will be excreted in the urine when volume and potassium deficits have been corrected.

Alkalosis Posthypercapnic Respiratory Acidosis

The response of the carbon dioxide/carbonic acid/bicarbonate/carbonate buffer system to the addition of carbon dioxide is to increase the plasma bicarbonate concentration. In addition, hypercarbia stimulates proton secretion by cells involved in renal acidification causing acid loss to the urine; hypercarbia also decreases blood pressure and glomerular filtration rate, which reduces the bicarbonate filtered load and supports an increased serum bicarbonate concentration.[8] All these events are part of metabolic compensation in

respiratory acidosis, and they serve to maintain blood pH at a level much nearer to normal than might be expected from the increase in carbon dioxide tension. Although the bicarbonate level is increased, this increase is not truly metabolic alkalosis.

When respiratory failure is corrected, the carbon dioxide tension may decrease rapidly. This is especially true in instances in which patients are placed on ventilator support. As the partial pressure of carbon dioxide decreases, serum pH increases and alkalemia may develop. The situation should be self-correcting. When the effects of respiratory acidosis on proximal bicarbonate reabsorption and on glomerular filtration have been removed, the resulting increase in filtered load and decrease in proximal reabsorption should lead to rapid excretion of excess bicarbonate. This process should occur within 48 hours of the restoration of normal ventilation. Within this period, the increased bicarbonate is expected, and little additional intervention is needed. However, in the clinical setting, the patient with respiratory failure often is given diuretics in an attempt to improve respiratory function. The result of this intervention is further contraction of the effective arterial volume, increase of aldosterone levels, and potassium loss. If this has occurred, the patient may be unable to undergo the anticipated bicarbonatriuresis. An extended period of metabolic alkalosis may ensue. Treatment is to restore volume and circulation, and to repair any potassium deficits that may have occurred.

We add 2 additional notes of caution. First, when one administers saline to a patient such as the one just described, one potential result is that the resulting bicarbonaturia serves to deliver a nonreabsorbable anion (bicarbonate is a nonreabsorbable anion in the presence of alkalemia) to a distal tubule and cortical collecting duct primed for potassium secretion by aldosterone. As a result, large amounts of potassium then may be lost in the urine, resulting in potassium deficiency, which renders the metabolic alkalosis chloride resistant. Second, the use of acetazolamide to induce bicarbonatriuresis will induce even greater potassium losses. The potassium deficits inherent in the posthypercapnic state must be corrected. Both volume and potassium are required in this condition.

Poststarvation Alkalosis

Refeeding carbohydrates after starvation is an unusual but well-documented cause of metabolic alkalosis. Mechanisms are not certain, but may include conversion of circulating ketoacids to bicarbonate after significant losses of ketoacids in the urine.[99]

Maintenance Phase of Metabolic Alkalosis

The preceding discussion has outlined both common and uncommon mechanisms by which metabolic alkalosis is generated. One of 2 processes occurs. Metabolic alkalosis is either generated by the net gain of alkali (as in the case of bicarbonate administration or calcium carbonate ingestion)

or by the net loss of acid (as in emesis/nasogastric suction or renal loss of acid during stimulation by mineralocorticoid hormones). When either of these processes occurs in the presence of normal or increased effective arterial volume, the resulting metabolic alkalosis is limited. Metabolic alkalosis tends to dissipate if its generating mechanism is interrupted and kidney function and volume status are normal.

The most common clinically relevant examples of metabolic alkalosis are marked not only by a generation phase, but also by the presence of a definable maintenance phase. Three major factors classically underlie the maintenance phase of metabolic alkalosis in most clinical situations. These are as follows: (1) changes in circulating volume (volume depletion), generalized hemodynamics (heart failure), and intrarenal hemodynamics (alteration of preglomerular and postglomerular resistance and filtration fraction) that combine to reduce the filtered load of bicarbonate traversing the proximal tubule despite the increase of plasma bicarbonate concentration; (2) increased aldosterone secretion, which occurs secondary to diminished volume and increased AII concentration, that stimulates renal acid secretion; and (3) potassium depletion and hypokalemia, which alter glomerular hemodynamics, stimulate the renal H,K-ATPase, and secondarily increase renal acid secretion in the presence of aldosterone. A terse summary of these effects could simply state that the regulation of bicarbonate concentration and pH is sacrificed to preserve volume and potassium stores. If volume and potassium balance are normal, metabolic alkalosis self-corrects.

The mechanisms by which metabolic alkalosis is maintained are regulated by the actions of several mediators. These are divisible into those classically recognized to be important to metabolic alkalosis, primarily renin, angiotensin, and aldosterone, and those more recently recognized, primarily endothelin and nitric oxide. The role of the renin-angiotensin-aldosterone axis in the maintenance of metabolic alkalosis has long been recognized and need not be reviewed in depth here. The stimuli for renin release include input from stretch-sensing renal baroreceptors, the composition of tubular fluid at the macula densa, central nervous system input, circulating or local prostaglandins, and cAMP-mediated circulating hormones. Decreased circulating volume, decreased salt delivery to the macula densa, and changes in central nervous system input all may act to increase renin release when volume is depressed. The primary result of increased renin release is enhanced production of angiotensin I from angiotensinogen (renin substrate). The conversion of angiotensin I to AII is mediated by converting enzyme. AII increases blood pressure by increasing arterial vasoconstriction, which increases postglomerular resistance, thereby increasing filtration fraction and glomerular filtration rate. It acts on the proximal tubule to increase bicarbonate reabsorption, and stimulates the adrenal gland to increase aldosterone release. Aldosterone in turn acts on the distal nephron to increase the apical membrane sodium conductance and the basolateral membrane Na,K-ATPase activity in sodium-reabsorbing principal cells and the H-ATPase of type A intercalated cells. This produces increased sodium absorption, in-

creased transepithelial potential, and directly increases proton secretion. The increase in transepithelial potential drives passive potassium secretion and enhances electrogenic urinary acidification medicated by the H-ATPase. Thus, through the actions of AII on the proximal tubule, and actions of aldosterone on the distal nephron, proximal bicarbonate reabsorption is stimulated, and distal proton and potassium secretion are enhanced. The renin-angiotensin-aldosterone axis irretrievably links the maintenance of volume to acid-base and potassium homeostasis. Newer aspects of angiotensin and aldosterone action and the actions of nitric oxide and endothelin are considered later.

Recent Developments Impacting on our Understanding of Metabolic Alkalosis

Angiotensin II

Our concepts regarding the diversity of AII effects have expanded markedly in the past decade. This comes in part from the recognition that all of the cellular machinery for local AII production and/or AII receptors exists in widely diverse tissues, including the heart, jejunum, liver, and lungs. It is now clear that the renal proximal tubule synthesizes AII and that AII receptors are found on both brush-border and basolateral membranes. In the S_1 segment of the proximal tubule AII binding is 10-fold higher than the S_2 segment; binding density in the pars recta (S_3) is minimal. All later tubule segments contain AII receptors, with the lowest density noted in the cortical collecting duct.

Type 1 angiotensin receptors mediate most tubular transport and hemodynamic effects of AII. In the proximal tubule Liu and Cogan[100] found that systemic infusion of AII, which did not change blood pressure, was associated with a 50% increase in bicarbonate reabsorption in the S_1 segment of the early proximal tubule and a significant, although lesser, increase in flux in the late proximal convoluted tubule. Subsequent studies have shown that AII can be released at the luminal membrane by perfused tubules and by cultured proximal tubular cells, documenting its importance as a regulator of transport in the renal cortex.[101,102] This apical membrane release is detectable despite the presence of large amounts of degrading angiotensinases in the apical brush border. Approximately 90% of an infused arterial dose of AII is degraded after a single pass through kidney.[103] Seikaly et al[102] further examined the importance of luminal membrane AII release in glomerular ultrafiltrate using micropuncture techniques and found intraluminal AII concentrations to be 1,000-fold higher than that in plasma.

The nonhemodynamic effects of AII in the kidney are many; some are more well known than others. In proximal tubular cells AII stimulates H^3-thymidine incorporation (hypertrophy), as well as actin and collagen synthesis, and it enhances the action of epidermal growth factor.[104,105] AII also directly stimulates the release of several peptides involved in

cell proliferation (hyperplasia) and fibrosis (progressive renal injury). These peptides include endothelin, platelet-derived growth factor, and transforming growth factor-β.[106] AII also stimulates ammoniagenesis, a phenomenon long associated with hypokalemia, and a further stimulus to renal hypertrophy. In acid-base balance, one thus could postulate that AII sets the scene for the unchecked maintenance of metabolic alkalosis. Coupled with its action to decrease urinary nitric oxide secretion (a potent renal vasodilator) and cause a decrease in glomerular filtration rate, these effects of AII act as a positive feedback and drive a relentless increase in bicarbonate retention.[107] Kwon et al[108] showed that if AII was given to rats for 7 days, apical NHE3 labeling was increased markedly in the medullary thick limb (inner stripe of the outer medulla) and in the proximal convoluted tubule. By contrast, Wall et al[109] showed that AII at 10^{-8} mol/L in the surrounding bath decreased acid excretion in the rat medullary collecting tubule.

The best-studied mechanism of AII action is its stimulation of aldosterone release from the zona glomerulosa of the adrenal cortex. Classic theory states that in response to a decrease in pressure, pH, or distal salt delivery to the macula densa, renin is released from the juxtaglomerular cells. Renin then enzymatically converts angiotensinogen to angiotensin I with the latter converted to AII. AII then stimulates the release of aldosterone. Hyperkalemia is also a potent stimulus to aldosterone release but probably not via the renin-AII mechanisms just described.[110] Given all the new information obtained using a variety of techniques such as knock-out and overexpression studies, molecular and microarray investigations, and human genetic profiling, it is time to reassess the classic theory. For 2.5 decades we have recognized the importance of autocrine and paracrine (cross-talk) elements within cells. We are now just beginning to discover the complexity of this issue with regard to AII.

Aldosterone

In the past decade new studies have uncovered several new biologic actions of the mineralocorticoid hormone aldosterone, some of which are nongenomic. These actions occur quickly, at times within minutes in the kidney and in nontraditional target tissues and will be considered here first. The full relevance of these new actions to hypokalemic metabolic alkalosis in human pathophysiology remains to be elucidated but these new data provoke significant questions.

Terada et al[111] identified aldosterone receptors in cultured rat mesangial cells and glomeruli and showed that aldosterone stimulates c-Raf and certain components of the cell-cycle pathway, notably cyclin D_1 and cyclin A promoter activities, as well as their protein and mitogen-activated kinases. These actions may adversely affect glomerular filtration rate with time, in a manner analogous to the deleterious changes induced by aldosterone in the heart, where the aldosterone antagonists (eg, spironolactone) have been proven to be clinically beneficial.

Quinkler et al[112] recently provided evidence for excess mineralocorticoid receptor abundance in human renal biopsy tis-

sues with a significant increase in the response of inflammatory mediators such as renal transforming growth factor-β1 messenger RNA (mRNA) (3-fold increase), interleukin-6 mRNA (2-fold increase), and macrophage chemoattractant protein-1 (7-fold increase). In renal tubular cells, Cha et al[113] found that macrophage chemoattractant protein-1, connective tissue growth factor, and nuclear factor-κ B were increased in abundance after incubation with aldosterone and that spironolactone pretreatment prevented this response. In rat renal fibroblasts, aldosterone treatment resulted in rapid phosphorylation of extracellular signal-regulated kinases 1/2 followed by a subsequent increase in collagen (types I, III, IV).[114] The spironolactone antagonist eplerenone blocked these actions (ibid).[114] Human mesangial cells[115] showed that AII stimulated aldosterone synthesis and that the addition of low-density lipoprotein synergistically increased the rate of synthesis.

In the medullary thick ascending limb 1 nmol/L aldosterone rapidly decreases bicarbonate reabsorption by 30%. This action was preserved when maneuvers were used to inhibit basolateral NHE1 activity. Similar results were obtained in medullary thick ascending limbs from NHE1 knockout mice. By using inhibitors to block an apical proton conductance and apical K-HCO$_3$ cotransport, Good et al[116] concluded that aldosterone must modulate the apical NHE3 exchanger.

In the collecting duct aldosterone increases Na reabsorption by stimulating basolateral Na,K-ATPase activity and the ENaC. These effects occur after 4 hours and result from gene transcription. In the cortical collecting duct proton secretion (H-ATPase) is stimulated in α-intercalated cells via a Na-dependent mechanism, whereas in the medullary collecting duct aldosterone action is Na-independent. Aldosterone does not appear to directly affect the collecting duct H,K-ATPase, however, the H,K-ATPase is stimulated to reabsorb potassium and to secrete protons during potassium-depleted states.[46]

In studies performed in our laboratory,[46] we showed that the most severe metabolic alkalosis occurred when rats given pharmacologic amounts of aldosterone were simultaneously placed on a low-potassium diet (see Table 3).[46] In a series of 9 groups (only 8 of which could be studied) we showed that in vivo chronic aldosterone excess (delivered via osmotic minipump) to animals on a low-potassium diet developed

Table 3 Effect of Body Potassium Stores and Plasma Aldosterone on Plasma HCO$_3$ (mEq/L) and Collecting Tubule H-ATPase and H, K-ATPase

|	Low-Potassium Diet	High-Potassium Diet
Zero aldosterone	21 mEq/L	16 mEq/L
	H-ATPase ~ 0	H-ATPase ~ 0
	H,KATPase ↑ ↑	H,KATPase ~ 0
High aldosterone	46 mEq/L	
	H-ATPase ↑ ↑ ↑	
	H,K-ATPase ↑ ↑	

Normal potassium diet, normal aldosterone. and sham groups not shown.[44]

the most severe metabolic alkalosis with a plasma bicarbonate averaging 46 mEq/L. When collecting tubules from these animals were dissected and the H-ATPase and H,K-ATPase activities were measured using radiolabeled ATP, this same group had the highest levels of activity of both enzymes. The lowest H-ATPase activity was found in the 3 groups given no aldosterone, regardless of whether they were on a low-, normal, or high-potassium diet. The highest activity of H,K-ATPase was found in the 3 groups of animals on the low-potassium diet, confirming the findings of Wingo[117] in the isolated perfused tubule. (In the low-potassium groups the H,K-ATPase activity was unaffected by the amount of aldosterone given via osmotic minipump.) Although these effects are thought to reflect events occurring in the intercalated cells of the distal nephron, the close association of intercalated cells to the Na-reabsorbing principal cells could allow regulators of sodium transport to influence the 2 enzymes in a paracrine fashion.

Rozansky[118] recently reviewed the aldosterone-sensitive pathways and the perturbations resulting within collecting duct Na-reabsorbing cells during aldosterone stimulation. From the effect of aldosterone to enhance basolateral surface area and Na, K-ATPase activity to the hormone's action to increase ENaC surface cell expression (and, in the late DCT to increase the thiazide-sensitive NaCl cotransporter), it is clear that many aldosterone-sensitive genes are involved and 3 to 4 secondary intracellular messengers are synthesized. Whether these act only within one cell type or are capable of subsequently acting on closely surrounding cells (or releasing yet other mediators) remains to be elucidated.

In Figure 1 the effect of aldosterone (lower right corner) on some of these intracellular actions is shown. Serum and glucocorticoid-inducible kinase 1 (SGK1) mRNA is stimulated within 30 minutes of incubation with aldosterone.[118] It subsequently phosphorylates specific serine and threonine residues, including, in the principal cells, those on the Nedd4 to 2 protein. This then allows ENaC to increase at the apical cell surface and hence stimulate Na reabsorption. SGK1 back-diffuses to the basolateral membrane and stimulates the Na,K-ATPase. SGK1 function requires activation by phosphatidylinositol 3 kinase, an enzyme activated by insulin and other mediators.[119,120] The WNK proteins, another family of serine-threonine kinases, also are stimulated by aldosterone, at least kidney-specific WNK1. This isoform may modulate SGK1. Other WNK isoforms may affect ROMK channels[121] in the Na-reabsorbing cells. Although Figure 1 outlines the intracellular effects of aldosterone on sodium reabsorption, comparable mechanisms may be involved in the proton secretory cells.

Gumz et al[122] has shown that early transcriptional effects of aldosterone include stimulation of preproendothelin in addition to SGK. By using microarray technology these investigators also report that a number of other transcripts are upregulated by aldosterone, including connective tissue growth factor, a prostaglandin E–receptor subtype, a retinoic acid–responsive transcript, and claudin-1. How these and other
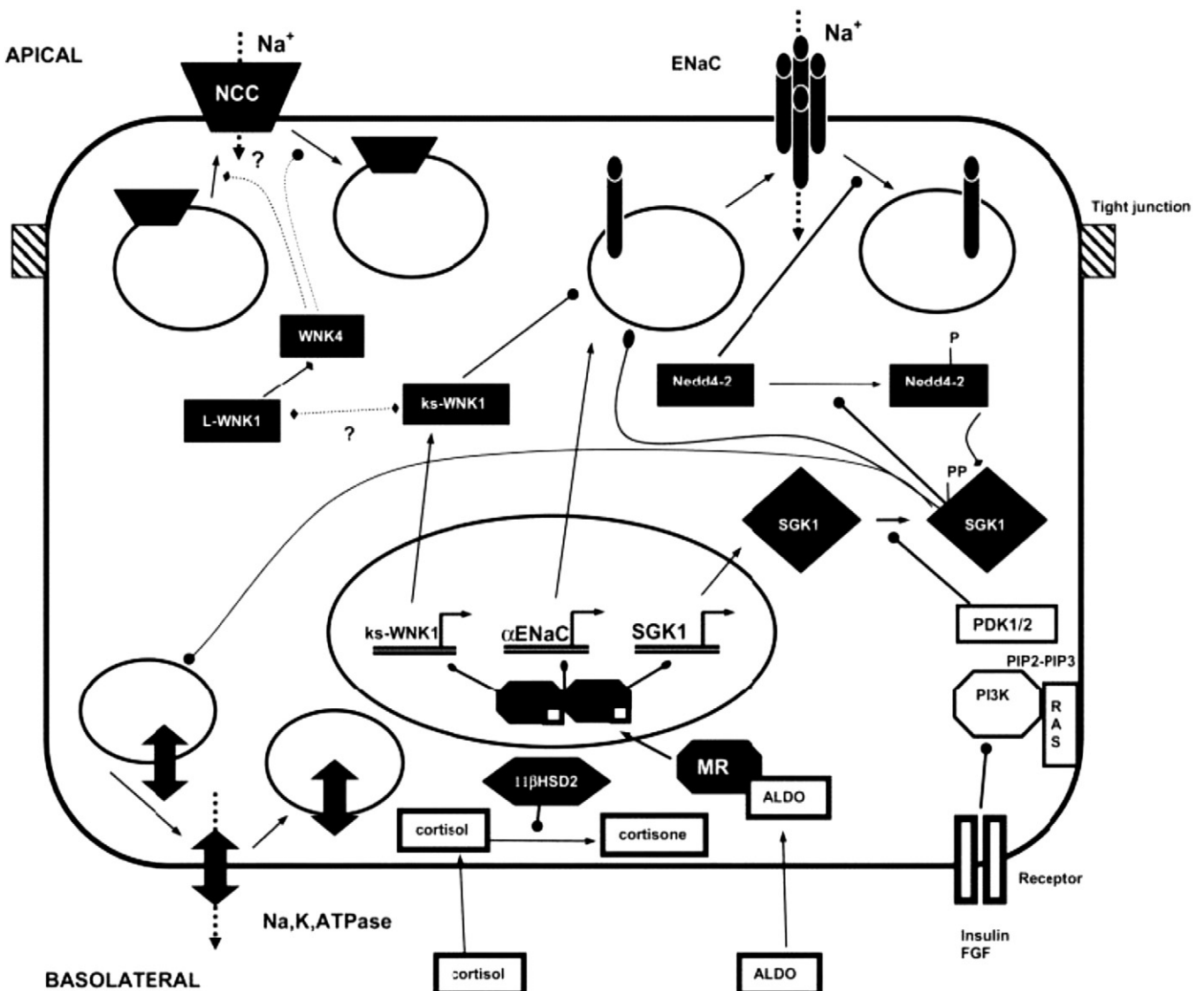
Figure 1 Regulatory mechanisms initiated by aldosterone (ALDO) in DCT2 cells to increase transepithelial sodium transport. Darkened shapes represent key signaling molecules of the pathway that are discussed in greater detail in the text. Pointed arrows represent movement of the molecules within the cell's nucleus, cytoplasm, or subsurface membrane vesicles. Circle-end arrows represent stimulatory mechanisms, diamond-end arrows represent inhibitory mechanisms. Stippled lines with nearby "?" indicate unresolved interactions. Dashed arrows indicate the direction of Na$^+$ reabsorption from the apical to the basolateral compartments. Reprinted with permission from Rozansky.[118]

factors influence metabolic alkalosis or its amelioration remains to be determined.

Endothelin

In the past 15 years the endothelins (ET) have been recognized to play an important role in both proximal and distal urinary acidification. This 3-member 21-amino acid polypeptide family (ET-1, ET-2, and ET-3) is synthesized in diverse cell types in a wide variety of tissues including vascular endothelial cells, heart, liver, gut, gonads, neurons, adrenal medulla, and the eye. ETs are best known for causing smooth muscle contraction (they are 30 times more potent than AII), but now it is clear that one or all are involved in stimulation of growth, myocardial fibrosis, and neural crest migration.

All 3 ETs (or their mRNAs or Binding Sites) Are Found in the Kidney

ET-1 is the best understood of the ETs. It is synthesized by the glomerulus, proximal tubule, the thick ascending limb, and the medullary collecting duct (outer and inner). ET-2 mRNA and protein have been detected in human proximal tubules; ET-3 has been found in the cortical collecting duct in addition to those sites noted earlier for ET-1.

ET-1 alters hemodynamics and decreases the glomerular filtration rate. ET-1 also stimulates Na reabsorption in the proximal tubule and inhibits Na reabsorption in the medullary collecting duct. Finally, ET-1 inhibits vasopressin-stim-

ulated water flux and Cl reabsorption in the medullary collecting duct.

One of the first reports on ET-1 and renal acidification is that of Eiam-Ong et al.[123] These investigators showed that ET-1 (10^{-8} to 10^{-11} mol/L) increased Na/H antiporter activity in rabbit brush-border membrane vesicles. They further showed that when basolateral membrane vesicles were incubated with ET-1, the Na:$3HCO_3$ cotransporter was stimulated. These important findings suggest that in the proximal tubule ET-1 stimulation of the apical membrane proton secretion and its attendant basolateral bicarbonate flux could act in metabolic acidosis to preserve acid-base homeostasis. It could just as well be involved pathophysiologically to maintain metabolic alkalosis (eg, under circumstances when ET-1 was released to produce intense vasoconstriction). It subsequently was shown by Guntupalli et al[124] that NHE-3 activity was enhanced in renal cortical slices by ET-1; similar results have been documented in cultured renal cells. A calcium-medicated mechanism has been suggested.[125,126]

The effects of the ETs are mediated by either the ET_A or ET_B receptor, both of which have domains characteristic of G-coupled receptors (ie, Ca-mediated). The receptors have significant homology; however, their binding affinities are such that ET-3 is an E_B-selective agonist whereas ET-1 is nonselective for both receptors. Brush-border membrane vesicles contain both receptors, but binding studies suggest that ET_B is found at higher abundance.

Chu et al[126] overexpressed both ET receptors in opossum kidney cells in culture and showed that after a 5-minute incubation with ET-1, those cells that overexpressed the ET_B receptor, increased NHE-3 activity by approximately 25%. This stimulation of proton flux was inhibited by the selective ET_B-receptor blocker BQ-788. In subsequent studies in mice proximal tubule suspensions, ET-1 and ET-3 enhanced NHE-3 activity. To examine this further they repeated the experiments in mice in which the ET_B receptor had been disrupted genetically and compared the results with those rescued by a transgene. (This was performed in a model of toxic megacolon showing the role of the ET_B receptor in mice.[127]) Laghmani et al[128] showed that ET-1 increased NHE-3 activity significantly in the rescued mice proximal tubules whereas in the receptor-deficient suspensions there was no such effect.

Precisely how ET-1 affects proximal acidification has been shown by Laghmani et al.[128] As stated earlier, one of their observations suggested that a Ca-mediated mechanism[126] was present, but that study did not elucidate the steps involved. In a series of elegant studies from their laboratory it appears that with a decrease in cell pH, the pH sensor praline-rich tyrosine kinase 2 is activated and this in turn activates c-Src by phosphorylation of tyrosine 416 in its catalytic domain. There is a downstream increase in c-fos/c-Jun expression and activator protein 1 activity. This then results in an increase in cortical cell preproET-1 mRNA (and a decrease in preproET-3 mRNA), and an increase in proET-1, followed by a stimulation in cellular ET-1. The interaction of ET-1 with the ET_B receptor phosphorylates and moves NHE-3 from its subapical localization in the intracellular pool to the plasma

Table 4 Factors Stimulating Endothelin

Volume contraction*
Hypokalemia*
Erythropoietin
Hypoxia/?hypercarbia*
Acute renal ischemia*
Increased renal medullary osmolarity*
Radiocontrast dyes
Cyclosporine
ET-secreting hemangioendotheliomal
Disease states
    Hypertension (some forms)
    Endotoxin shock*
    Congestive heart failure*
    Cardiogenic shock*
    Atherosclerosis
    Progressive glomerulopathies
    Hepatorenal syndrome
Humoral mediators
    AII*
    Arginine vasopressin
    Insulin
    Thrombin
    Interleukin-1
    Transforming growth factor-b
    Tumor necrosis factor
    Bradykinin

*Factors that may generate or maintain metabolic alkalosis.

membrane. This involves cytoplasmic microfilaments and is blocked by latrunculin B, an inhibitor of microfilament organization and the actin cytoskeleton. There appears to be no role for protein kinase A, protein kinase G, cyclo-oxygenase, lipoxygenase, or cytochrome P450 pathways; there is some controversy over the role of protein kinase C.[126,128] Importantly, there is a link between the ET_B receptor and nitric oxide formation in the kidney.

A second major mechanism of proton secretion, and hence bicarbonate reabsorption in the proximal tubule, is the vacuolar H-ATPase. As discussed, roughly one third of acidification occurs via this enzyme. There is no information supporting a direct action of endothelin on the vacuolar H-ATPase. Doubtless, information regarding the role of endothelin will be forthcoming with the transgenic models described earlier.

Table 4 summarized some of the factors known to stimulate the synthesis and release (or action) of endothelin. Of particular interest here and to the possible generation and/or maintenance of metabolic alkalosis are volume contraction, hypokalemia, hypoxia/hypercarbia, renal ischemia, medullary osmolarity, congestive heart failure, and shock, as well as AII. Although the triggering mechanisms may seem obvious in most cases (ie, an absolute or effective decrease in arterial volume), there are some disease states in which the mechanism stimulating ET-1 release is not so clear.

During hypokalemia, the marked decrease in muscle blood flow and subsequent tissue anoxia probably releases endothelin from those vascular beds. Renal ischemia results in ET release from mesangial cells of the glomerulus, the

same site thought to be involved in the hepatorenal syndrome and in chronic progressive glomerulopathies.

Precisely how increased medullary tonicity results in ET release is not clear. It should be noted that nitric oxide, atrial natriuretic peptide, and prostacyclin inhibit ET action, either by affecting synthesis or release.

In the distal nephron, from the bend of the loop of Henle to the medullary collecting duct, far less is known about ET-1 and its role in the maintenance of metabolic alkalosis. The mouse thick ascending limb contains the apparatus for endothelin biosynthesis and receptors for ET-1 action. In both medullary and cortical thick limb 1×10^{-8} MET-1 (the threshold was 1×10^{-13} mol/L) added to either the lumen or the bath significantly inhibited net chloride flux (J_{Cl}) by 33%.[129] This inhibition was partially reversible with time and was abolished completely with the addition of BQ-788, indicating the effect was via the ET_B receptor. ET-1 did not affect basal or vasopressin-stimulated cAMP content; and prostaglandin inhibition, as assessed in the presence of 3×10^{-6} mol/L ibuprofen, did not alter the effect of ET on J_{Cl}. ET-1 did not increase cytosolic Ca concentration in the mouse thick limb, whereas it did after other maneuvers, including 1×10^{-8} mol/L AII. The action of ET-1 on J_{Cl} appears to be related to protein kinase C activation in that diacylglycerol reproduced the effect.[129] These findings suggest a role for ET-1 on salt transport in the thick ascending limb and may contribute to natriuresis.

The medullary thick ascending limb also absorbs bicarbonate via an apical membrane Na/H antiporter NHE-3.[130] Good et al[131] showed that the isolated perfused medullary thick ascending limb vasopressin inhibits bicarbonate reabsorption via a cAMP-mediated mechanism. Hyperosmolality also inhibits bicarbonate reabsorption. By contrast, hypoosmolality stimulates medullary thick limb bicarbonate reabsorption. This occurs via phosphatidylinositol 3-kinase because inhibitors of this pathway (ie, wortmannin and LY294002) completely block the effect.[131] Such a phosphatidylinositol 3-kinase mechanism has been shown for Na/H antiporter activity induced by epidermal growth factor in intestinal epithelia cells.[131] Should ET-1 affect thick limb bicarbonate transport, one could predict it would stimulate the bicarbonate transport in this segment.

In isolated tubule studies the various segments typically are bathed and perfused in a solution containing 25 mmol/L HCO_3, equilibrated with 95% O_2–5% CO_2 (pH 7.45), with highly stylized chemicals. It seems unlikely that the thick limb sees this concentration of bicarbonate in the intact mammal under most circumstances. It is difficult at this time to place a defect in thick ascending limb bicarbonate transport into a pathophysiologic schema because there is no disease resulting in bicarbonate waste from this site. The 2 examples known to occur, administration of loop diuretics and Bartter syndrome, stimulate urinary acidification. Indeed, giving furosemide to human beings with metabolic alkalosis makes the alkalosis worsen, whereas acetazolamide administration ameliorates it.

In the distal convoluted and connecting tubules, ET-1 increases acidification in both control rats and those given

80 mmol/L $NaHCO_3$ drinking solution for 7 to 10 days to induce chronic metabolic alkalosis and a maneuver known to reduce distal acidification. In these in vivo micropuncture studies the effect of ET-1 in both groups of animals occurred without a significant change in plasma pH or partial pressure of carbon dioxide. In the control animals ET-1 primarily decreased distal tubule proton secretion whereas in the alkalotic animals the polypeptide decreased HCO_3 secretion. This latter finding lends support to a role of ET in the maintenance of metabolic alkalosis, particularly if its release is stimulated by one or more of the factors listed in Table 4.

In the collecting tubule, the 2 major enzymes involved in the maintenance of metabolic alkalosis are the vacuolar H-ATPase and the P-type H,K-ATPase. Stimulation of either or both of these enzymes would result in bicarbonate regeneration and loss of protons into the tubular lumen. There are no direct data regarding the effects of ET on the biochemical activity of these 2 important enzymes.

Nitric Oxide

Nitric oxide (NO) is metabolized from L-arginine by 1 of 3 nitric oxide synthase (NOS) isoform enzymes, all of which have been found in the kidney.[132] Inducible NOS (iNOS) differs from the other 2 forms in that it binds calmodulin at resting cytosolic Ca concentrations, and the gene encoding it is found on chromosome 17. There is functional evidence for iNOS action in the proximal tubule after inflammatory insult. The endothelial NOS gene is found on chromosome 7, and its protein has been found in proximal tubule, thick limb, and collecting duct in addition to the endothelial lining the arterioles (afferent and efferent) and vasa recta. Neuronal NOS, whose gene is localized to chromosome 12, has been localized to the collecting duct and the macula densa, and is expressed after maneuvers that stimulate inflammation. The precise regulators within the kidney that determine which of the NOS isoforms is turned on or off are not yet known.

All of the renal isoforms can be inhibited by endogenously produced dimethyl arginines. These compounds are increased in chronic renal failure and in states associated with endothelial dysfunction (eg, hypertension, heart failure, atherosclerosis). They can prevent the diverse actions of NO, the best known of which is vasodilation. Excessive production of NO appears to contribute to a number of inflammatory states, including tubulointerstitial disease, glomerulonephritis, obstructive uropathy, and transplant rejection.

Both neuronal NOS and iNOS play a role in solute and fluid transport in the kidney. In neuronal NOS knockout mice, proximal bicarbonate reabsorption and fluid transport are decreased and metabolic acidosis results[133]; in iNOS knockout mice, a decrease in $JHCO_3$ and water reabsorption of one-third has been noted.[134] Neither of these effects was found in endothelial NOS knockout mice. Application of NO to proximal tubules has been shown to stimulate $JHCO_3$ and water flux and to directly inhibit Na/H antiporter activity[135] and Na,K-ATPase activity.[136,137] There is no information as to the effect of NO on the vacuolar H-ATPase in proximal tubule.

In the distal nephron, NO inhibits bicarbonate reabsorption probably by a direct effect to block both the apical Na/H exchanger and the Na-K-2Cl cotransporter.[138-140]

Tojo et al[141] showed that in the collecting duct NO inhibits the H-ATPase in intercalated cells. This finding, all other things being equal, would result in impaired proton secretion and the tendency to mitigate the maintenance of metabolic alkalosis and decrease the action of aldosterone. It would be of interest to examine H-ATPase activity throughout the entire nephron in the 2 knockout mice just described. In addition, the interaction of NO with endothelins and Ang II should be examined carefully because the latter plays a significant role as regards to aldosterone action, and, hence, the maintenance of metabolic alkalosis.

Treatment

In the vast majority of patients, the treatment of metabolic alkalosis has been relatively constant for decades. If there is evidence of volume depletion, whether in terms of total body volume or effective arterial blood volume, the treatment requires that the deficit be repaired. In practice, this most often means the administration of normal saline. There have long been debates about whether it is the sodium or the chloride that leads to a decrease in proximal bicarbonate reabsorption, and whether chloride delivery or volume is responsible for the effect, but the clinician's answer is the same: saline should be given in metabolic alkalosis with evidence of volume depletion. This group of patients includes those with persistent emesis or nasogastric suction, the milk alkali syndrome, and other forms of volume depletion.

When the patient is compromised by congestive heart failure and suffers from a decrease in effective arterial blood volume while suffering an excess of total body salt and water despite the use of diuretics aggressive enough to produce metabolic alkalosis, care must be exercised if saline is given. Indeed, the point of treatment will generally be to improve cardiac output by some other means that produces significant improvement in renal blood flow, and changes filtration fraction and glomerular filtration rate. Similar situations may exist in the nephrotic individual who has compromised circulation as a result of intravascular depletion while again suffering from peripheral fluid overload, and more rarely, in the cirrhotic patient with ascites who has been diuresed too aggressively. More often, these individuals have developed metabolic alkalosis as a result of the effects of diuretics and will be better treated by the provision of potassium chloride because their prior treatments will have more likely than not rendered them potassium deplete.

Posthypercapnic metabolic alkalosis usually presents a combination of alkali overload, some degree of volume depletion, and, most often, serious potassium depletion. Careful volume expansion may be needed, but more often, the key is potassium supplementation.

The major new developments in the treatment of metabolic alkalosis involve the treatment of metabolic alkalosis in the case of Liddle syndrome, in which instance the knowledge that the mechanism of disease is an abnormally open

sodium channel directs therapy. Amiloride or triamterene may be used to inhibit the channel; spironolactone offers no benefit. Bartter and Gitelman's syndromes remain difficult to treat. One must provide salt and water to restore volume, replace potassium chloride, and, in the case of Gitelman's syndrome, provide magnesium, but the overall response is most often disappointing. Amiloride, triamterene, and spironolactone may be used as adjuncts in Bartter syndrome because there is secondary hyperaldosteronism and subsequent potassium and proton secretion in the collecting duct.[142,143] However, these drugs do not alter the potassium loss in the loop and DCT related to the inherited or acquired defects in the NaK2Cl, ROMK, and CCl2, transporters. Angiotensin-converting enzyme inhibitors also have been used to prevent the stimulation of aldosterone release, but use is limited by hypotensive effects.[144,145] The observation that prostaglandins are increased has led to the use of nonsteroidal anti-inflammatory drugs with some success.[146] Similarly, in Gitelman's syndrome some success has been achieved by the addition of inhibitors of collecting duct sodium reabsorption.[147] The use of the other adjuncts mentioned with regard to Bartter syndrome may be tried.

Conclusion

Has the Bench Caught up to the Bedside?

As a result of the work of nephrologists, biochemists, and molecular biologists, we now know the precise and varied causes of Bartter syndrome, Gitelman's syndrome, and Liddle syndrome, but only in Liddle syndrome has our knowledge led to better treatment (amiloride). Considerable knowledge has certainly been added in regard to the diagnosis and treatment of abnormalities of steroid metabolism. Nevertheless, if one were to transport the Robert Pitts of 1950 to the present day and confront him with a case of a patient with metabolic alkalosis caused by gastrointestinal losses or the use of diuretics, the most common causes of the disorder, he would doubtless be able to diagnose the condition, outline its causes, and recommend the administration of normal saline and potassium for its correction. The contemporaries of Dr. Kurtzman would similarly be able to offer a fully rationalized explanation of the effectiveness of the treatment based on an understanding of the basic relationship of the reabsorption of volume and bicarbonate in the proximal tubule, and the effect of aldosterone and sodium delivery in the distal nephron. Much has been added to define the fine points of these interactions and to explain the basic mechanisms of reabsorption along what is now viewed as a much more complex nephron, but the original insights remain correct and continue to serve medicine well.

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